



Eight Commonly Used Pesticides and Clothianidin Interact Potentially Toxic in Binary and Ternary Manner on Honey Bees (*Apis Mellifera*)

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Abstract

Although though honey bees (*Apis mellifera*) have been the subject of several toxicological assessments, the majority of this research has only looked at the impacts of specific substances. But in the field, honey bees are frequently exposed to chemical combinations. In this work, we used a feeding approach to assess the effects of eight different pesticides on honey bees, including clothianidin (CLO), carbaryl (CAR), thiodicarb (THI), chlorpyrifos (CHL), gamma-cyhalothrin (GCY), beta-cyfluthrin (BCY), and spinosad (SPI).

Keywords: Toxicology; Honey bees; Pesticides

Introduction

The use of pesticides remains one of the main pest management strategies in modern agriculture. However, the unintentional misuse of pesticides in farming areas can pose certain risks to the environment and non-target organisms, such as bees. Honey bees (*Apis mellifera*) play a major role in the maintenance of plant biodiversity and food security through agricultural productivity [1].

Methods

Morphology of *Apis mellifera*

The honey bee (*A. mellifera*) queenright colonies that we employed in our research were originally bought from beekeepers that were situated close to the Mississippi cities of Magee and Perkinston in pine forest and pasture settings. At a solitary bee yard in the Mississippi Wildlife Management Area, bee colonies were established (North Stoneville, MS, USA). Frames with sealed brood covering more than 50% of them were moved into a lab incubator with no light and a temperature of 33.0 ± 0.5 °C [2, 3].

Neonicotinoids

In this investigation, nine pesticides from seven chemical classes were chosen, including CLO (Belay 50 WDG, Valent), CAR (Sevin XLR Plus, Bayer CropScience), and THI (Larvin 3.2F, Bayer); CHL (Lorsban 4E, Dow AgroSciences); BCY (Baythroid XL 1 EC, Bayer); GCY (Declare, Cheminova); TET (Domark 230 ME (Steward EC, DuPont). Instead of using these pesticides' active ingredients, commercial formulations were used because our goal was to imitate field circumstances and assess the possible interaction toxicities of chemical combinations to honey bees using formulations typically used in field applications [4,5].

Test evaluating mixture toxicity

CLO was binarily and ternarily combined with the other eight pesticides in order to uncover any potential interacting effects of pesticide combinations on honey bees. Using honey bee workers subjected to sucrose solutions containing CLO and other compounds, the toxicities of chemical combinations were evaluated. In parallel testing, we directly contrasted the toxicity of the individual drugs and their mixtures. Honey bees were exposed to repeated dilutions of each chemical with a fixed equitoxic constant mixture ratio (the same toxicity impact from each chemical according to individual 4-day LC50 values) in order to evaluate the combined effects of pesticide combinations.

A geometrical ratio was used to test six dilutions of various pesticide combinations. The contents of each chemical were varied, but the above-mentioned ratios in the combinations kept the same in order to establish the complete concentration-response correlations. There were 20 honey bee workers (4 days old) in each of the three cages used in the experiment for each tested level. The other methods for analysing mixtures followed similar steps to those applied to analysing pesticides individually [6, 7].

Discussion

The initial stage in assessing the possible risk of pesticides to honey bees is typically acute toxicity studies (Spruill et al., 2019). Recent research has demonstrated that CLO is a highly lethal insecticide for honey bees, with a 2-day LC50 value of 0.077 (0.05-0.11) g a.i. mL⁻¹ when exposed by feeding (Laurino et al., 2011). According to some studies, CHL has a 10-day feeding toxicity (LC50) of 0.58 g a.i. mL⁻¹ and a 2-day feeding toxicity (LC50) of 2.29 (0.63-1.29) for *A. mellifera*. According to our findings, IND also demonstrates considerable toxicity, with a 2-day LC50 value of 3.54 (3.28-3.82) g a.i. mL⁻¹ in a prior investigation [8].

Conclusions

Of the nine pesticides studied, CLO was the one that was the most hazardous. Most of the binary combinations of CLO and other pesticides had interactions with *A. mellifera* that were both additive and antagonistic. Except for CLO+TET+THD, all ternary compounds including CLO and TET had synergistic effects on honey bees. This study showed that pesticide combinations may be more hazardous to honey bees than individual pesticide tests. Assessments of the potential environmental risks based on individual exposure may therefore not properly safeguard agricultural pollinators. The synergistic effects of

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pesticide mixtures should receive more consideration because they can seriously endanger natural ecosystems [9, 10].

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Declaration of Competing Interest

The authors affirm that they have no known financial or interpersonal conflicts that would have appeared to have an impact on the research presented in this study.

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